

# STUDIES ON THE DIFFERING EFFECTS OF TUMOR NECROSIS FACTOR AND LYMPHOTOXIN ON THE GROWTH OF SEVERAL HUMAN TUMOR LINES

JEFFREY BROWNING<sup>1</sup> AND ANN RIBOLINI

From the Department of Cell Biology and Immunology, Biogen Inc., 14 Cambridge Center, Cambridge, MA 02142

The relative ability of TNF and lymphotoxin (LT) to inhibit the growth of five human tumor cell lines was examined both in the presence and absence of IFN- $\gamma$ . Two adenocarcinoma lines, HT-29 and SK-CO-1, were 20- and 320-fold more sensitive to the inhibitory effects of TNF than LT in 3- to 4-day proliferation assays. In contrast, the breast carcinoma line BT-20 showed only a one- to twofold difference. The MCF-7 and ME-180 cell lines exhibited intermediate behavior. These results parallel the reported disparate potencies of TNF and LT in their effects on endothelial cells, hematopoietic development and their abilities to sustain a mixed lymphocyte response. Radiolabeled TNF binding studies showed two classes of receptors ( $K_d$  0.04 to 0.15 nM and 0.2 to 1.0 nM) on the highly sensitive SK-CO-1 line. HT-29 cells also appeared to possess some high affinity-binding sites, whereas the BT-20 line completely lacked the high affinity form. Thus the presence of high affinity-binding sites correlated with increased sensitivity to the antiproliferative effects of TNF. Cold TNF competed with the binding of radiolabeled human TNF three- to fivefold better than LT for binding to all three lines. These relatively small differences between the TNF and LT receptor-binding characteristics are insufficient to explain the dramatic disparity in their antiproliferative properties. Likewise, the absolute concentrations of the unlabeled cytokines necessary to block the binding of  $^{125}\text{I}$ -TNF were 10- to 150-fold higher than the levels necessary to elicit the biologic response. Thus, the receptor binding data conflict with the growth inhibitory effects. This discrepancy is discussed in terms of either separate receptors for TNF and LT or more complex phenomena such as receptor cooperativity possibly resulting from multivalent interactions with the trimeric form of TNF.

TNF and LT<sup>2</sup> also called TNF- $\alpha$  and TNF- $\beta$ , respectively, are two proteins characterized originally for their cytolytic properties and, in the case of TNF, its *in vivo* tumor necrotic activity (for reviews see References 1 to 5). These

proteins can be directly cytolytic to certain tumor lines, yet in other cases they can actually promote growth (6, 7). TNF is produced by many cell types including monocytes, T cells, NK cells (1, 9), and even cells of nonhematopoietic origin (8, 10), whereas LT appears to be released primarily by lymphocytes (2, 11-13). More recently, the cloned gene products have been demonstrated to display a wide range of activities. TNF appears to play major roles in specific aspects of metabolic control, the response to endotoxin shock (14), the control of hematopoietic cell development (15-18), and it shares with IL-1 many proinflammatory actions (e.g., Reference 19). Although many of these activities have also been demonstrated for LT, a comprehensive picture of their relative roles remains unclear.

Initially TNF and LT were described as having similar cytotoxic activities (20), however, more recent work has indicated that LT is less active on certain tumor lines (21, 22). A comparison of LT and TNF activities reveals disparate potencies in several systems. The human endothelial cell responds to TNF stimulation by secreting IL-1 and CSF, by expressing surface adhesion proteins, and by exhibiting increased adherence for neutrophils (23, 24). Similarly, granulocyte CSF release from fibroblasts was induced differently by LT and TNF (25). In each of these cases, LT was at least 100-fold less active than TNF. In our experiments, TNF was 20- to 50-fold more effective than LT in its ability to induce primary human umbilical vein endothelial cells to bind retinoic acid-induced HL-60 cells (J. Browning and P. Lawton, unpublished results). Likewise, LT is less effective relative to TNF in inducing human peripheral monocytes to release CSF-1 (18) and in its ability to promote the mixed lymphocyte response (26). In contrast to this pattern, LT has been reported to cause an inflammatory reaction in normal skin whereas TNF was inactive (27). In receptor-binding experiments with ME-180 (28), L929 (29, 30), HL-60 (31), and U937 (32, 33) cells, LT competed with labeled TNF almost equally well for binding to its receptor and vice versa, suggesting that both molecules interact with the same or similar receptors. The conflicting nature of the observations that these proteins share common receptors yet display widely differing activity profiles poses an interesting problem.

In this paper, we examined the anti-proliferative effects of TNF and LT on several human tumor lines both in the presence and absence of the synergistic factor, IFN- $\gamma$  and have attempted to correlate differing biologic activities with receptor-binding properties. The apparent discrepancy between the receptor-binding studies and the biologic effects is discussed.

Received for publication January 25, 1989.

Accepted for publication June 19, 1989.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup>Correspondence and reprint requests should be addressed to Jeffrey Browning, Biogen, 14 Cambridge Center, Cambridge, MA 02142.

<sup>2</sup>Abbreviations used in this paper: LT, lymphotoxin; MTT, 3(4,5-dimethylthiazol-2-yl)2,5 diphenyl tetrazolium bromide; EGGS, ethylene glycol bis(succinimidylsuccinate); CHO, Chinese hamster ovary.

## MATERIALS AND METHODS

**Cytotoxins.** Human rTNF was expressed in *Escherichia coli* and purified to homogeneity as previously described (34). This preparation had a specific activity of  $5.3 \times 10^7$  U/mg as defined by reference to the National Biological Standards Board TNF (Hertfordshire, England) in the 1-day L929 cell cyolytic assay. Early experiments with murine rTNF utilized protein produced at Biogen, whereas later experiments employed material that was a gift of Suntory Pharmaceutical Co. (Osaka, Japan). Both preparations had a specific activity of  $3 \times 10^7$  U/mg in the L929 assay system. A cDNA clone encoding human LT was isolated from a RPMI 1788 cDNA library by using an exact oligonucleotide probe to the sequence previously described (20). The gene was stably transfected into CHO cells and amplified by using a dihydrofolate reductase/methotrexate selection system. rLT was purified from serum-free, LT-transfected CHO cell-conditioned media with a series of Sepharose S, lentil lectin, and FPLC Mono Q column chromatography steps. rLT purified by this route was 90 to 95% pure, was glycosylated, and had a specific activity of  $3.1 \times 10^7$  U/mg by reference to the standard TNF sample. The protein content of the TNF and LT samples was determined by parallel amino acid composition analysis. Aliquots of cytotoxins at 1 (LT) and 10 (TNF)  $\mu\text{g}/\text{ml}$  were frozen and fresh aliquots thawed for each assay. Human rIFN- $\gamma$  was produced from *E. coli* with a specific activity of  $1.5 \times 10^7$  U/mg (encephalomyocarditis virus/WISH cell system). Murine rIFN- $\gamma$  was produced in *E. coli* yielding a preparation with a specific activity of  $1 \times 10^7$  U/mg by using a vesicular stomatitis virus/L929 assay system.

**Cytolytic and cytostatic assays.** The murine tumor line L929 and the human lines HT-29, SK-CO-1, BT-20, ME-180 and MCF-7 were obtained from the American Type Culture Collection (Rockville, MD) and grown in RPMI 1640 supplemented with glutamine, 50  $\mu\text{M}$  2-ME, penicillin/streptomycin, pyruvate, 10 mM HEPES buffer, and 10% Hyclone FCS (L929, HT-29, and ME-180 lines) or MEM (Eagle's) supplemented as above only without HEPES and with nonessential amino acids (SK-CO-1, BT-20, and MCF-7 lines). The cells were free of mycoplasma contamination (Gen-Probe test) and periodically monitored. One-day cyolytic assays using murine L929 cells were carried out by preplating L929 cells in 96-well plates (0.05 ml/well) before use. When the cells were about 50% confluent (1 to 2 days), serial dilutions of TNF or LT in 0.05 ml with 2  $\mu\text{g}/\text{ml}$  mitomycin C were added. Growth was quantitated by using mitochondrial reduction of the dye MTT as described (35). After 20 to 30 h, 0.01 ml of 5 mg/ml MTT dye was added, and after an additional 3 to 4 h, 0.1 ml of isopropanol with 10 mM HCl was added to dissolve the reduced MTT dye. The OD at 570 nm was determined with an ELISA plate reader. Cytostatic assays of 3- to 4-day duration were performed as described for the cyolytic assay except that mitomycin C was omitted. Typically, wells were seeded with 3000 to 8000 cells/well at day 0, which resulted in a 0.4 to 0.8 OD signal after 3 to 4 days of growth. The initial cell concentration was adjusted to compensate for the slow growth of lines such as SK-CO-1 and BT-20. After 3 to 4 days, growth was assessed by using the MTT dye reduction method as described above. Although the absolute concentration of cytotoxin required to give 50% inhibition varied slightly from experiment to experiment, the potency of LT relative to TNF was very reproducible. In both the growth-inhibition assays and the receptor-binding studies, a monomeric m.w. was assumed for both TNF and LT.

**TNF-binding assays.** rTNF was iodinated by the Iodogen method as described (28) except that the following ratios of reactants were used: 2  $\mu\text{g}$  Iodogen, 10  $\mu\text{g}$  TNF, and 1 mCi of  $^{125}\text{I}$  in a volume of 0.05 ml.  $^{125}\text{I}$  was reacted with Iodogen alone for 2.5 min at 0°C followed by the addition of TNF and the reaction continued for 10 min. The free Iodine was separated on a BioRad P-6DG column. Bioassay using L929 cytotoxicity showed typically retention of at least 50% of the starting activity yielding a specific activity of 12 to 20  $\mu\text{Ci}/\mu\text{g}$  active TNF. SDS-PAGE and autoradiography showed that only TNF was iodinated. For direct binding experiments, cells from nonconfluent plates were removed with 5 mM EDTA in calcium/magnesium-free PBS, collected and resuspended at a concentration of 0.5 to  $2 \times 10^7$  cells/ml by repeated passage through a 5-ml pipette into RPMI 1640 with 10% FCS and 0.1% sodium azide. Aliquots of 0.5 ml were rocked in a 3-ml conical polypropylene tube for 4 h at 4°C in the presence of  $^{125}\text{I}$ -TNF. Nonspecific binding was assessed by including 4  $\mu\text{g}/\text{ml}$  cold TNF in control samples and it constituted generally 20% or less of the total binding. The cells were washed twice with 3 ml of ice-cold Dulbecco's PBS with 5% FCS and 0.1% sodium azide and counted. Direct binding was analyzed with a standard Scatchard plot. Competitive binding was analyzed in a similar fashion by using 50 pM  $^{125}\text{I}$ -TNF in the case of HT-29 and SK-CO-1 cells and 100 pM with the L929 cells. The same unlabeled TNF and LT solutions used in the growth inhibition assays were employed in the competition assays. Competitive binding was analyzed by using logit analysis

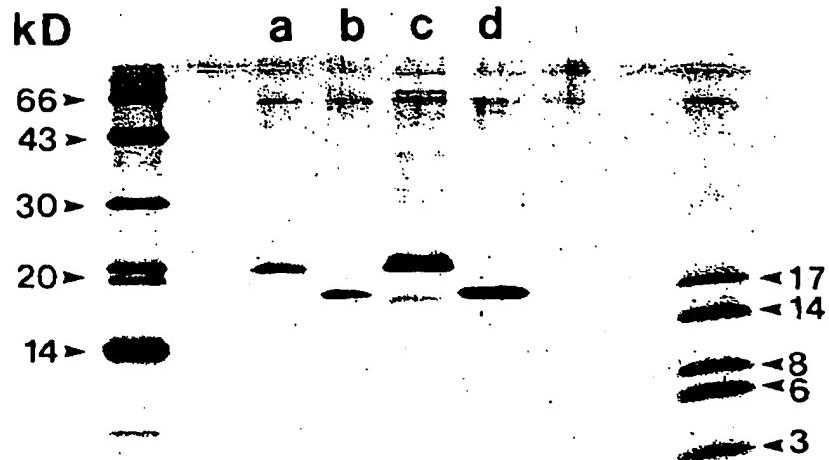
whereby  $\text{logit} = \ln(Y/1-Y)$  and Y is equal to percent bound (36). In all of the receptor-binding analyses, curves were fitted by eye and no attempt was made to deconvolute curvilinear Scatchard plots by more rigorous methodologies.

**TNF/LT cross-linking.**  $^{125}\text{I}$ -TNF or  $^{125}\text{I}$ -LT (prepared as described above for TNF) was diluted into 50 mM potassium phosphate buffer, pH 7.4, with 100  $\mu\text{g}/\text{ml}$  BSA and with or without various detergents. Samples at final concentrations of 100 (TNF) and 50 (LT) ng/ml were treated at 0°C with 1 mM EGS (Pierce Chemical Co., Rockford, IL; a concentrated stock solution was prepared in dry dimethylformamide) or at room temperature with glutaraldehyde (Electron Microscopy Sciences, Ft. Washington, PA) at a final concentration of 0.1% v/v. After 30 min, SDS-gel sample buffer was added and the samples were boiled and analysed by SDS-PAGE and autoradiography.

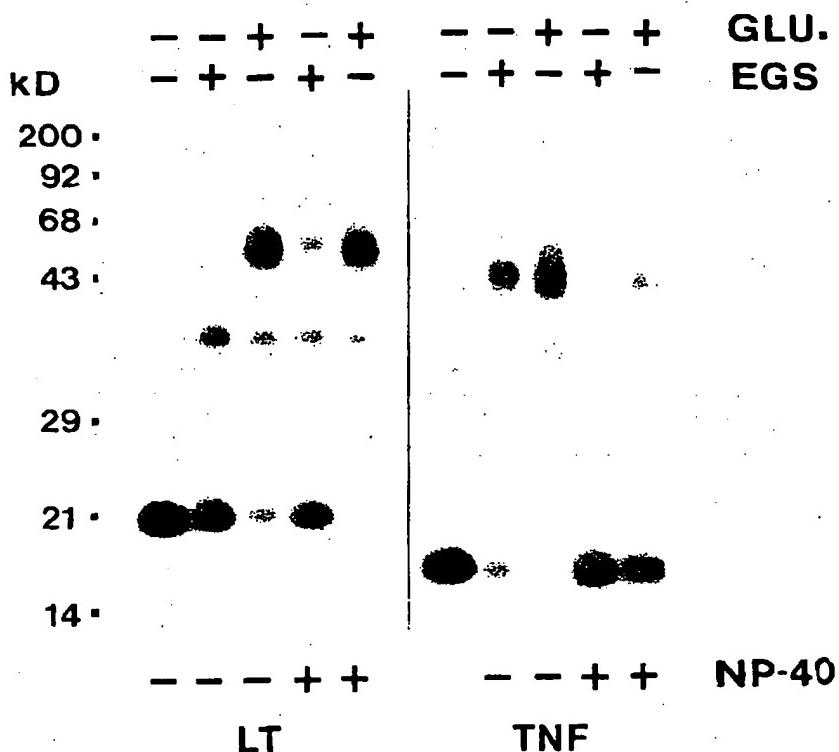
## RESULTS

**Properties of CHO-derived human lymphotxin.** The amino acid sequence of human LT as determined from the cDNA nucleotide sequence agreed completely with the previously published sequence (20). Because the properties of rLT secreted from CHO cells have not been well described, we have partially characterized this protein. On the basis of lentil lectin binding, the bulk of the secreted LT was glycosylated. The major rLT species migrated in SDS PAGE at an apparent  $M_r$  of 20 kDa (see Fig. 1), which is greater than the 18-kDa nonglycosylated form produced in *E. coli* (20), yet smaller than the 25-kDa species originally purified from the human B-lymphoblastoid line, RPMI 1788 (37). The amino acid composition of the purified protein agreed with the expected composition. Initial batches of rLT contained two minor species of slightly higher of  $M_r$ . N-terminal amino acid sequencing from polyvinylidene difluoride blotted (38) samples of all three forms revealed identical N termini corresponding to the N terminus of the 25-kDa form described by Aggarwal et al. (37). The different forms probably result from heterogeneous glycosylation. A set of LT-like proteins can be immunoprecipitated from  $^{35}\text{S}$ -methionine-labeled, phorbol ester/PHA-stimulated PBL with a rabbit antiserum raised against the purified CHO cell-derived LT (unpublished observations). In this case a ladder of presumably different glycosylated forms was observed and the predominant form secreted from CHO cells corresponded to the smallest form produced by peripheral lymphocytes. The major species released by lymphocytes migrated at 25 kDa. In an examination of three HIV-infected human T cell lines, three different LT sizes were observed (39). Apparently, most of the natural lymphocyte-derived LT is more heavily glycosylated than CHO-derived rLT. A SDS-PAGE gel comparing recombinant *E. coli*-derived TNF with rCHO cell-derived LT is shown in Figure 1. The LT preparation was 90 to 95% pure with the major impurity being a 17-kDa protein corresponding probably to the 20-kDa form described by Aggarwal et al. (37).

TNF exists as a compact trimer in solution (40, 41). Superose S6 gel exclusion FPLC chromatography in 0.1 mg/ml BSA, 0.2 mM EDTA and PBS showed that both human and murine rTNF migrated with an apparent  $M_r$  of about 45 kDa. CHO cell-derived human rLT also migrated with a  $M_r$  of 45 kDa in this system. The iodinated preparations of TNF used in the binding studies co-migrated with unlabeled TNF. Cross-linking studies were carried out to establish more rigorously whether LT is a trimer. Figure 2 shows the results of EGS and glutaraldehyde cross-linking of iodinated TNF and LT. EGS was capable of almost completely cross-linking TNF into a



**Figure 1.** SDS polyacrylamide gel comparison of human rLT and human rTNF. Lanes were loaded with 0.2 µg (a, b) or 1.0 µg (c, d) of LT (a, c) or TNF (b, d). Gel was composed of 16% acrylamide/0.49% bis acrylamide and run according to the system described by Schaegger and Jagow (63) and stained with Coomassie blue. Bands at 68 kDa represent fingerprint artifacts.



**Figure 2.** Reduced SDS polyacrylamide gel analysis of either EGS or glutaraldehyde (GLU.) cross-linked preparations of iodinated LT and TNF. Cross-linking was performed in the presence or absence of 1% NP-40. Shown is an autoradiograph of the gel. The m.w. standards were Bethesda Research Laboratory-prestained standards and the indicated sizes are the values as assessed by running these markers alongside the markers used in Figure 1.

trimeric structure and with LT both dimeric and trimeric forms were observed. LT is cross-linked less efficiently by EGS. Glutaraldehyde was more effective than EGS in cross-linking both TNF and LT and both glutaraldehyde-treated cytokines were found as trimers with no indications of larger aggregates. TNF cross-linking was completely blocked by inclusion of 1% NP-40 in agreement with the reported disruption of the TNF trimeric structure by detergents (41). In contrast, the trimeric structure of LT is stable to detergent treatment. The cross-linking of LT by both EGS and glutaraldehyde was actually enhanced by addition of either 1% NP-40 or 0.1% SDS (never warmed beyond room temperature). Thus LT is a very stable trimer, even at concentrations approaching physiologic levels, and this result is in agreement with the trimeric m.w. reported for natural RPMI 1788-derived LT (42).

**Specific activities of LT and TNF for cytotoxicity and cytostasis of L929 cells.** A comparison of TNF and LT in both 1- and 3-day murine L929 proliferation assays is shown in Figure 3. LT was slightly less potent than TNF on a molar basis in the 1-day assay with 50% lysis observed at 28 to 33 pM for LT and 13 to 15 pM for TNF. On the other hand, LT was more effective in the 3-day assay (Fig. 1 and Table I) similar to results previously reported (43). The reasons for the increased potency in the 3-day assay are not clear. In the 3-day L929 antiproliferative assay, the potency of TNF also increased about twofold. The specific activity for this preparation of LT is similar to the values determined for both natural and *E. coli*-derived recombinant material as assessed by using the 1-day assay (20), thus glycosylation of the protein by the CHO cell does not affect the activity as was found previously for natural and *E. coli*-derived recombinant

**Figure 3.** Comparison of recombinant human LT (○—○) and recombinant human TNF (●—●) in 1-day cytolytic (A) and 3-day anti-proliferative (B) assays using the murine fibrosarcoma L929. In the cytolytic assay about 20,000 cells/well were present, whereas in the 3-day assay, wells were seeded with 3,000 cells/well. Growth was quantitated with the MTT reduction system and relative growth is presented as OD<sub>570</sub> nm. Dotted lines at the top of the panels represent the levels of growth observed in the absence of added rTNF or rLT.

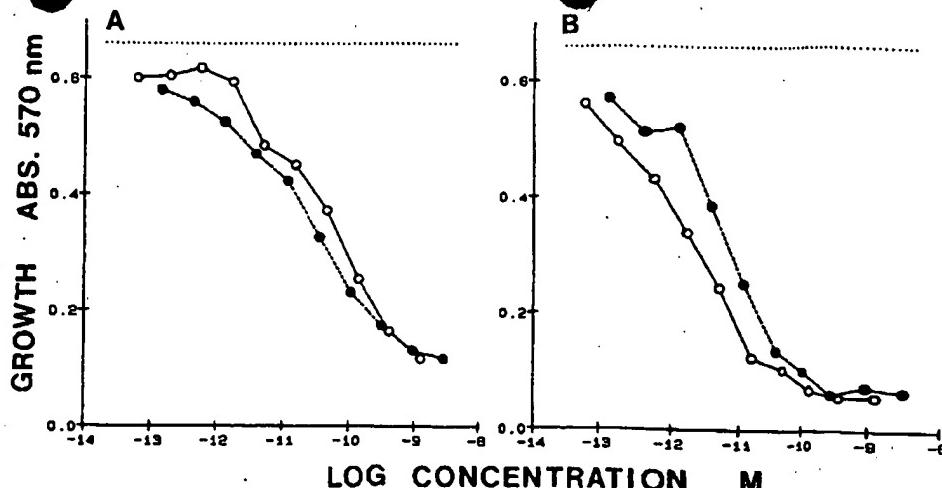


TABLE I

Effects of LT and TNF on the growth of several tumor lines

Cell Line	Expt. No.	Concentration (pM) Giving 50% Growth Inhibition			
		-IFN-γ		+IFN-γ*	
		LT	TNF	LT	TNF
L929 (1-day) <sup>b</sup>	1	33	13	35	12
	2	26	15		
	3	28	14		
L929 (3-day)	1	3	12		
	2	1	4		
	3	3	8		
HT-29	1	>2500 <sup>c</sup>	>5000	610	31
	2	>2500	>5000	372	16
SK-CO-1	1	>2500	>5000 <sup>d</sup>	299	1
	2	>2500	>5000	300	2
BT-20	1	667	368	179	137
	2	415	285	139	106
ME-180	1	1250	184	215	13
	2	2500	552	240	33
MCF-7	1	>2500	2000	>2500	2000
	2				

<sup>a</sup> Murine IFN-γ was used in the case of L929 cell assays, human IFN-γ was used in all other cases. Both IFN were added at a concentration of 500 antiviral U/ml at the beginning of the assay.

<sup>b</sup> One-day cytolytic assay as opposed to 3-day growth assays in all other cases.

<sup>c</sup> The use of the greater than symbol indicates that the 50% point was not reached at this concentration.

<sup>d</sup> TNF stimulated growth in the absence of IFN-γ.

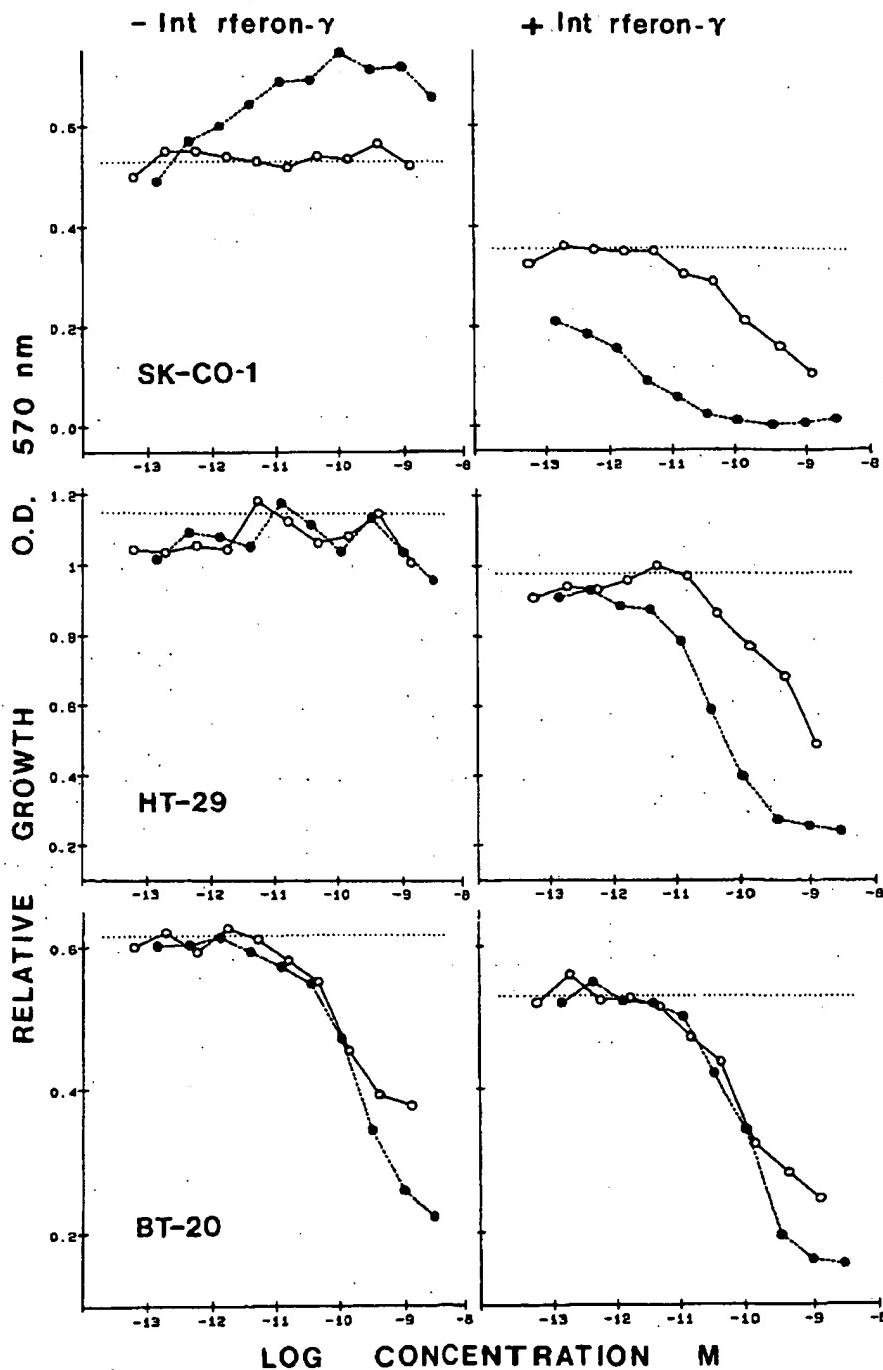
forms (20).

**Specific activity of LT and TNF for cytostasis of several human tumor lines.** IFN-γ synergizes with TNF and LT in antiproliferative assays (44–46). The effects of TNF and LT on the growth of five TNF-sensitive human tumors was examined both in the presence and absence of IFN-γ. The results of three such comparisons are shown in Figure 4 and summarized in Table I. The tumors differed dramatically both in the absolute sensitivity to TNF and LT as well as in the relative potency of these two proteins. For example, HT-29 cells have been described as being very sensitive to TNF in the presence of IFN-γ (46, 47) as was observed in these studies. In the absence of IFN-γ, HT-29 cells are unaffected by even very high levels of TNF. We had noticed during an earlier investigation of the lymphotoxin-like cytotoxins pro-

duced by the RPMI 1788 and the GM 3104 cell lines that what appeared to be natural lymphotoxin had a low specific activity on IFN-γ-treated HT-29 cells. This observation was confirmed with CHO-derived rLT in that rLT was about 20 to 25 fold less potent than TNF both on a molar basis and in terms of L929 LU. The SK-CO-1 line behaved in a fashion similar to the HT-29 adenocarcinoma in that IFN-γ was absolutely required for antiproliferative activity of LT or TNF. IFN-γ alone exhibited slight antiproliferative effects on the HT-29, SK-CO-1, and BT-20 lines at the 500 U/ml level. In the absence of IFN-γ, TNF acted as a growth factor on SK-CO-1 cells, an observation typically made in fibroblastoid systems (6, 7). Thus IFN-γ changes the way this cell interprets the signals generated by TNF, i.e., from enhanced growth to growth arrest responses. One could speculate that the antiproliferative action of IFN-γ is a result of an altered response to growth factors provided by the serum in a manner analogous to the TNF effects on the SK-CO-1 line. The antiproliferative activity of TNF was 200 to 320 times more potent than that of LT with this cell line. Although the degree of growth stimulation by TNF in the absence of IFN-γ was small at least as quantitated with the MTT assay, the concentration dependence paralleled closely that of the cytostatic activity observed in presence of IFN suggesting that IFN-γ does not modulate the affinity of the TNF receptor. A lack of receptor-affinity modulation by IFN-γ has been reported in other systems (46, 47). We attempted to block the growth inhibition of SK-CO-1 by 1 pM TNF with 50 to 100 nM LT. LT alone at these concentrations did not affect growth and its inclusion with TNF did not block the TNF activity. Hence, LT did not act to antagonize the low level TNF effect.

The breast carcinoma line, BT-20, was responsive to both LT and TNF in the absence of IFN-γ (IFN-γ being only slightly synergistic) and TNF was only one- to two-fold more potent than LT. The cell line ME-180, a cervical carcinoma, exhibited intermediate behavior with some synergistic interaction with IFN-γ and a 20-fold higher potency of TNF over LT. The breast carcinoma line, MCF-7, in our hands was relatively unresponsive to TNF with no synergy with IFN-γ. The 50% point for growth inhibition by LT for four of these lines remained in the 200- to 400-pM range. In contrast, the 50% point for TNF effects varied between 1 and 150 pM.

**Receptor-binding studies.** Scatchard analyses of di-



**Figure 4.** Comparison of the antiproliferative activity of rLT (○—○) and rTNF (●—●) on the growth of three human tumor lines: SK-CO-1, HT-29, and BT-20. Wells were seeded with 8000 (SK-CO-1 and BT-20) and 4000 (HT-29) cells/well and growth was assessed after 3 days (HT-29 and SK-CO-1) or 4 days (BT-20). Cultures were set up either with or without 500 U/ml of IFN- $\gamma$ . Dotted line signifies the level of growth observed in the absence of rTNF or rLT, either with or without IFN- $\gamma$ .

rect  $^{125}\text{I}$ -TNF-binding experiments are shown in Figure 5 and summarized in Table II. In these studies, the concentration of active TNF was quantitated by using the L929 bioassay and specific activities were calculated, assuming that both inactive and active molecules were labeled uniformly. In one case the self displacement method was used to assess the sp. act. and a roughly similar value was obtained (48). The L929 cell line bound 2500 to 5500 molecules of human TNF per cell with a dissociation constant of 0.5 to 1.0 nM, a value that agrees well with previously published results (30, 49). In one set of experiments, the Scatchard plots had clearly defined concave downward appearances when data at low TNF concentrations were included (data not shown). Such plots can

be indicative of positive cooperativity (50). Scatchard analysis of TNF binding to SK-CO-1 cells revealed two types of binding sites, a low affinity form with 3000 to 6000 sites per cell and a high affinity form with 1000 to 2000 sites/cell. The dissociation constant of the high affinity form,  $K_d$  0.07 to 0.15 nM, was 5- to 10-fold higher than that of the low affinity form,  $K_d$  0.2 to 1.0 nM. The HT-29 Scatchard plot was curvilinear, probably a result of contributions by both a low affinity site similar to that observed on the SK-CO-1 line and some higher affinity components. The number of receptor sites increased slightly with a 6-h pretreatment with IFN- $\gamma$ , however, these increases were not observed with 24- to 48-h exposure paralleling previous reports (46, 47). The BT-20

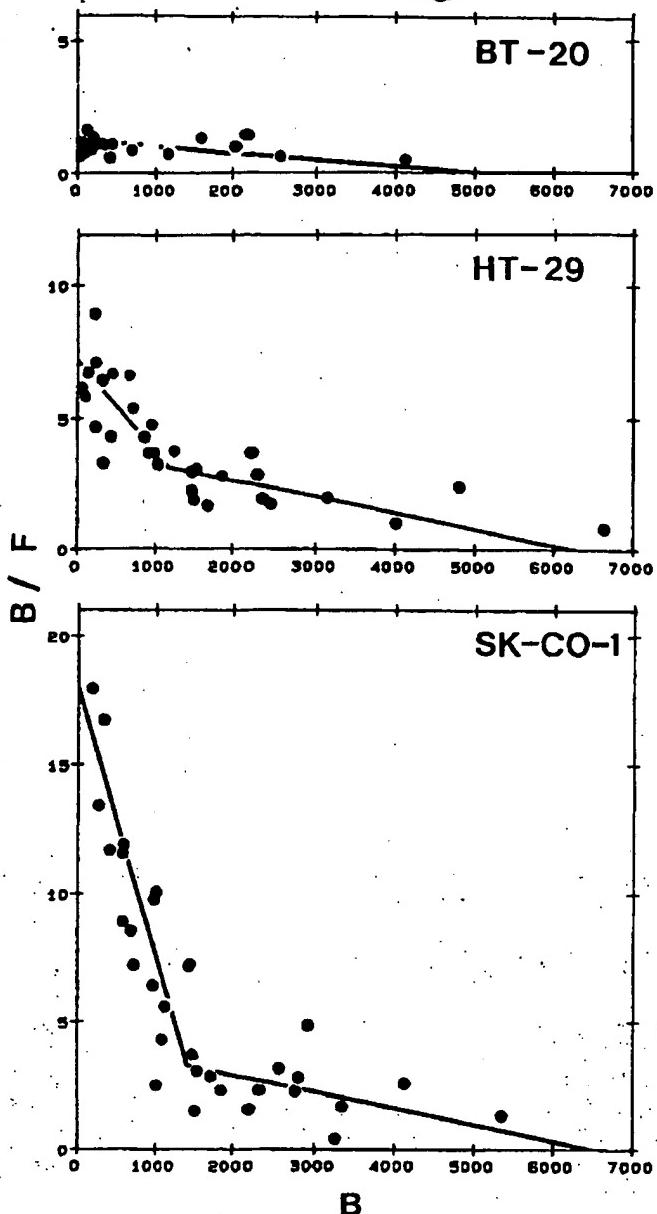


Figure 5. Scatchard analyses of the direct binding of  $^{125}\text{I}$ -TNF to three human tumor lines.  $B$  represents number of TNF molecules per cell. Shown are data pooled from several experiments, and in each case the cells had been treated with  $\text{IFN-}\gamma$  for 6 to 48 h.

TABLE II  
Summary of Scatchard analyses of direct  $^{125}\text{I}$ -TNF binding to various tumor lines

Cell	-IFN- $\gamma$		+IFN- $\gamma^a$	
	Sites/cell	$K_d$ (nM) <sup>b</sup>	Sites/cell	$K_d$ (nM)
L929	2500-5500	0.61-0.80		
BT-20			3000-5000	3.30-6.50
HT-29	2000-3300	0.25-1.00	1100-6000	0.40-1.10
SK-CO-1				
Low <sup>c</sup>	3000	0.46	3000-6500	0.20-1.00
High	1500	0.07	600-2000	0.04-0.15

<sup>a</sup>Pretreatment with 1000 U/ml  $\text{IFN-}\gamma$  for 6 or 48 h.

<sup>b</sup>Data represent the range of all experiments.

<sup>c</sup>Indicative of the high and low affinity sites found with this cell line.

line possessed the lowest affinity receptors and clearly lacked any high affinity receptor forms. Thus increased sensitivity to the biologic effects of TNF correlated with the presence of apparently higher affinity receptors.

Logit plots of pooled data from competitive binding experiments are shown in Figure 6 and the results of individual experiments summarized in Table III. Figure 6 represents data from both multiple cell preparations and different iodinated TNF lots. Both LT and TNF competed with labeled TNF binding to all the cell types examined. In the case of the murine L929 cell, human LT was similar to murine TNF and both were more effective than human TNF. Whether this increased binding of LT over TNF simply reflects aspects of the species barrier or implies that the L929 receptor is actually a LT receptor is not clear. In contrast to the murine cell line, LT was three- to fivefold less effective than TNF in competing with labeled TNF binding to the human HT-29 and SK-CO-1 lines. The BT-20 line possesses relatively low affin-

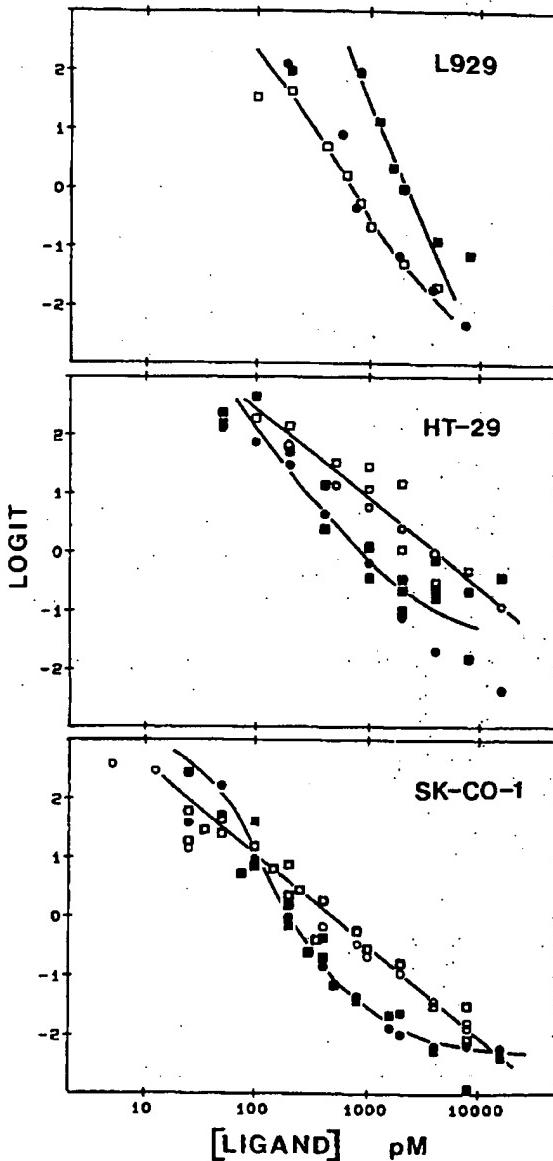


Figure 6. Logit analysis of the competition of unlabeled human rLT (open symbols) or unlabeled human rTNF (closed symbols) with  $^{125}\text{I}$ -human TNF for binding to murine L929, human HT-29, and human SK-CO-1 cells. In the experiments with HT-29 and SK-CO-1 cells, data from several experiments have been pooled and different symbols were used to denote whether the cells were pretreated with  $\text{IFN-}\gamma$  (squares) or were untreated (circles). In the L929 cell panel, the closed circles represent competition by unlabeled murine rTNF and the cells were not exposed to  $\text{IFN-}\gamma$ .

TABLE III  
Summary of competitive binding experiments

Cell Line	Concentration of Unlabeled Ligand (pM) at <sup>a</sup> Half-Maximal Inhibition			
	-IFN- $\gamma$ <sup>b</sup>		+IFN- $\gamma$	
	TNF	LT	TNF	LT
L929	2.30	600		
BT-20			~6,000	~30,000
HT-29	1.000	3,000	1,000	2,000
SK-CO-1	180	350	180	450

<sup>a</sup> Concentration of unlabeled TNF or LT required to displace one-half of the bound  $^{125}\text{I}$ -TNF from the cells.

<sup>b</sup> The cells were incubated with or without IFN- $\gamma$  for 6 h before performing the binding assay.

ity receptors and although accurate data could not be obtained, clearly much higher levels of TNF and LT were required to displace labeled TNF from receptor sites on this cell. Interferon treatment had no effect on the ability of cold ligand to displace labeled TNF, again agreeing with the lack of modulation of receptor affinity by IFN. In these experiments, the relative binding affinities of TNF for these lines as defined by the 50% inhibition point roughly paralleled the antiproliferative effects, however, the concentrations were generally at least 10-fold higher than the level yielding 50% of the biologic effect. In a classic analysis of various agonists competing for the same receptor, the binding curves can be shifted reflecting unequal receptor affinities, yet they should be parallel. In this logit analysis, the TNF curve for the SK-CO-1 line was found to be nonlinear and had a different slope when compared with the LT binding curve. The quality of the HT-29 data do not allow one to define accurately the curve shape. This observation indicates that the interactions of these two ligands with the receptor are not identical. Looking at the TNF-binding curve with the SK-CO-1 line (Fig. 6), one could surmise that the curves were roughly parallel at high ligand concentrations, yet deviate in the low concentration regions. The nonlinearity in the TNF curve may reflect the receptor heterogeneity seen in the Scatchard analysis. It should be noted that radiolabeled TNF binding studies may be plagued by an additional problem. Iodination by methods such as iodogen that yield high specific activity labeling do lead to TNF inactivation when the labeling is allowed to continue for longer times (J. Browning, unpublished observations). It is possible that individual subunits of the TNF trimer may be inactivated without serious loss of biologic activity, i.e., less than 50 to 70% losses. These partially defective yet more highly iodinated TNF molecules may exhibit abnormal binding in Scatchard analyses.

#### DISCUSSION

We have shown in this study that TNF and LT have different antiproliferative properties on several human tumor lines. The concentration of LT necessary for a half maximal antiproliferative effect remained in the range of 200 to 400 pM for three tumor lines, whereas the TNF concentrations required for similar activity varied over two orders of magnitude. With few exceptions, the presence of only one class of TNF-binding site has been demonstrated with dissociation constants ranging from 1 to 2000 pM depending upon the cell type. Unlabeled TNF and LT have been shown to compete with labeled

TNF for binding to its receptor (28, 30) and vice versa, i.e., cold TNF could compete with labeled LT binding to its receptor (29, 31, 32). Typically, LT and TNF were almost equally potent in this respect. The simplest model accommodating these data is the existence of a single receptor that can bind both LT and TNF. If one assumes that the biologic response is directly linked to receptor occupancy and that only a single type of TNF/LT receptor is present on these cells, it is difficult to explain the differing biologic effects of TNF and LT. In this case, the differing sensitivities to TNF may simply reflect the varying levels of signal transduction required to produce an effect, e.g., only 0.1 to 1% occupancy of the TNF receptors on a SK-CO-1 cell may be sufficient to trigger cytostasis. However, if LT is behaving as a simple TNF agonist, it too should trigger the biologic event at proportionately lower concentrations with a cell line like SK-CO-1. Because the potency of LT remained relatively unaltered in these different cells, more complex phenomena need to be considered.

Two possible explanations for this discrepancy have occurred to us. In the first case, both low and high affinity receptors for TNF exist with LT binding to a low affinity receptor with a  $K_d$  of about  $10^{-10}$  to  $10^{-9}$  M, but not to the high affinity form ( $K_d$   $10^{-10}$  to  $10^{-12}$  M). In this model, signal transduction results from occupancy of either receptor form, however, the increased sensitivity of certain cell types is a consequence of TNF binding to the high affinity-receptor form. The high and low affinity forms may reflect either different states of the same receptor or different receptor molecules. In the second model, TNF binding to its receptor occurs with some form of positive cooperativity possibly stemming from its trimeric structure. The apparent high affinity binding would represent actually a high avidity binding mode of a common low affinity receptor. In this model, LT would be incapable of interacting in a cooperative fashion. Whether cooperative interactions occur would depend probably not only on the receptor density, but also on other factors such as cytoskeletal linkages, etc.

TNF-binding studies were undertaken in an attempt to resolve this dilemma and these results suggest that elements of both models may contribute. SK-CO-1 cells and probably HT-29 cells were found to possess a high affinity form of the TNF receptor correlating with the increased sensitivities of these lines to TNF. High levels of LT, that alone were inactive, were unable to affect the low level anti-proliferative effects of TNF with the SK-CO-1 line suggesting that LT cannot bind to the high affinity form. Furthermore, one could speculate that the high affinity form may be the biologically relevant TNF receptor and hence LT may not elicit TNF-like activities under physiologic conditions.

On the other hand, TNF was only three- to fivefold better than LT in competing with  $^{125}\text{I}$ -TNF binding to the SK-CO-1 receptor in contrast to the 150- to 100-fold difference in biologic potencies. Thus the existence of a high affinity TNF receptor may explain the increased sensitivity of a cell type such as SK-CO-1 to TNF, however, the LT binding properties do not fit with its decreased biologic potency. It is possible that the competitive binding studies are complicated by the existence of a heterogeneous receptor population. Although most studies have not detected high affinity TNF binding, there are

a number of exceptions. High affinity TNF binding ( $K_d$  1 to 5 pM) was observed with human m nocytes (51) and HL-60 cells (16, 52) and in the latter case the presence of the high affinity form correlated with increased TNF potency. Likewise, high affinity receptors with  $K_d$  in the 1 to 5 pM range for murine TNF were described in the mouse L cell (53). Intermediate affinity receptors in the range of 20 to 70 pM have been described on endothelial cells, lymphocytes, and the breast carcinoma line MCF-7 (23, 54-56). Evidence for multiple TNF receptors also comes from consideration of the species specificity of receptors on several murine cell lines. The murine L929 cell responds almost equally well to both human and murine TNF, yet the murine CT6 T cell line (57), the mouse/rat T cell hybridoma PC-60 (58), and the murine line PG-19 (59) respond to murine TNF but not to human TNF. Whether these phenomena reflect separate LT and TNF receptors is not clear.

The analysis of ligand binding to multiple receptors can be very complex, nonetheless, the data obtained in this study suggest an additional possibility. The logit plots of TNF and LT competition for labeled TNF binding should yield parallel lines if the two ligands interact in a similar manner with the same receptor albeit with different affinities. The lack of parallel plots may indicate some form of receptor cooperativity. Trimeric TNF could undergo multivalent interactions with TNF receptors leading to a high avidity TNF interaction. This interaction could allow for biologic effects at low concentrations despite low to moderate affinity TNF binding to its receptor. In this model, LT must be considerably less effective in forming multimeric interactions with this receptor form. In support, it was found that monomeric TNF was biologically less active and bound with a much lower affinity than trimeric TNF (41). Conversely, one study employing iodinated monomeric *E. coli* derived-human LT observed relatively high affinity binding to the L929 cell receptor (29). Although most TNF cross-linking studies have indicated the existence of a 55- to 75-kDa receptor chain, the molecular nature of the TNF receptor remains presently unresolved. Immunoaffinity-purified TNF receptor was found to undergo ligand-dependent oligomerization, possibly reflecting binding of one TNF trimer with several receptor molecules (60). An additional 138-kDa protein was associated with TNF-responsiveness in the MCF-7 carcinoma (54) and the possibility that this species resulted from TNF cross-linked dimeric forms should be considered. Resolution of these aspects awaits the cloning and characterization of the TNF/LT receptors.

LT and TNF pose a problem arising in other apparently redundant systems such as IL-1 types  $\alpha$  and  $\beta$  and IFN- $\alpha$  and - $\beta$ ; namely, why do multiple forms of these cytokines exist? Although a region in the C-terminal domain that appears to be critical for receptor binding is relatively conserved (61), the amino acid sequences of LT and TNF are only 28% homologous overall (1, 2). The genes lie within 2 kb of each other (1), apparently the result of a gene duplication event, yet they are independently regulated (12, 13). Other than the disparate activities in the endothelial cell and monocyte systems and the antiproliferative effects described here, the qualitative biologic spectra of LT and TNF are quite similar. It is possible that the actual role of LT lies in an as yet undescribed

system and relies on interaction with a LT-specific high affinity receptor. It was in this light that several groups have reintroduced the suggestion that LT may be a mediator of delayed type hypersensitivity reactions (26, 62). Whether nature enjoys the evolutionary security of these redundant cytokines or exploits individual activities via separate receptors remains an open question.

**Acknowledgments.** The authors would like to thank the following people for their contributions to this work: Jerry Carson and C. Hession for isolation of the cDNA clone, J. Devlin for preparation of the RPMI 1788 RNA, R. Rothenbuhler and E. Kawashima for DNA sequencing, R. Moova for various expression constructs, J. Travernier and W. Fiers for generating the LT-transfected CHO line, E. P. Chow and E. Greco for protein sequence analyses, and B. Pepinsky and C. Ware for helpful advice.

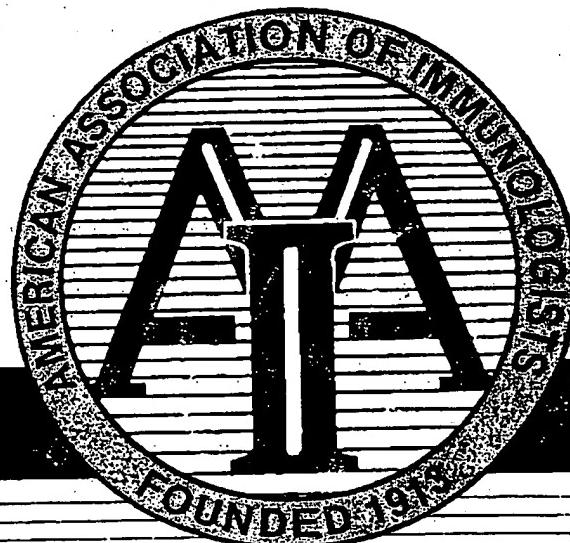
#### REFERENCES

1. Goeddel, D. V., B. B. Aggarwal, P. W. Gray, D. W. Leung, G. E. Nedwin, M. A. Palladino, J. S. Patton, D. Pennica, H. M. Shepard, B. J. Sugarman, and G. H. W. Wong. 1986. Tumor necrosis factors: gene structure and biological activities. *Cold Spring Harbor Symp. Quant. Biol.* 51:597.
2. Paul, N. L., and N. H. Ruddle. 1988. Lymphotoxin. *Ann. Rev. Immunol.* 6:407.
3. Old, L. J. 1985. Tumor necrosis factor. *Science* 230:630.
4. Beutler, B., and A. Cerami. 1988. Tumor necrosis, cachexia, shock and inflammation: a common mediator. *Ann. Rev. Biochem.* 57:505.
5. Sherry, B., and A. Cerami. 1988. Cachectin/tumor necrosis factor exerts endocrine, paracrine and autocrine control of inflammatory responses. *J. Cell Biol.* 107:1269.
6. Sugarmann, B. J., B. B. Aggarwal, P. E. Hass, E. S. Figari, M. A. Palladino, and H. M. Shepard. 1985. Recombinant human tumor necrosis factor- $\alpha$ : effects on proliferation of normal and transformed cells *in vitro*. *Science* 230:943.
7. Vilcek, J., V. J. Palombella, D. Henriksen-Destanfano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto. 1986. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med.* 163:632.
8. Spriggs, D. R., K. Imamura, C. Rodriguez, E. Sariban, and D. W. Kufe. 1988. Tumor necrosis factor expression in human epithelial tumor cell lines. *J. Clin. Invest.* 81:455.
9. Turner, M., M. Londei, and M. Feldmann. 1987. Human T cells from autoimmune and normal individuals can produce tumor necrosis factor. *Eur. J. Immunol.* 17:1807.
10. Rubin, B. Y., S. L. Anderson, S. A. Sullivan, B. D. Williamson, E. A. Carswell, and L. Y. Old. 1986. Nonhematopoietic cells selected for resistance to tumor necrosis factor produce tumor necrosis factor. *J. Exp. Med.* 164:1350.
11. Nedwin, G., L. P. Sverdovsky, T. S. Bringman, M. A. Palladino, and D. V. Goeddel. 1985. Effect of interleukin-2, interferon- $\gamma$  and mitogens on the production of tumor necrosis factors  $\alpha$  and  $\beta$ . *J. Immunol.* 135:2492.
12. Cuturi, M. C., M. Murphy, M. P. Costa-Gomi, R. Weinmann, B. Perussia, and G. Trinchieri. 1987. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. *J. Exp. Med.* 165:1581.
13. Sung, S.-S. J., J. M. Bjorndahl, C. Y. Wang, H. T. Kao, and S. M. Fu. 1988. Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. *J. Exp. Med.* 167:937.
14. Cerami, A., and B. Beutler. 1986. Cachectin: the dark side of tumor necrosis factor. *Cold Spring Harbor Symp. Quant. Biol.* 51:625.
15. Roodman, G. D., A. Bird, D. Hutzler, and W. Montgomery. 1987. Tumor necrosis factor-alpha and hematopoietic progenitors: effects of tumor necrosis factor on the growth of erythroid progenitors CFU-E and BFU-E and hematopoietic cell lines K562, HL-60 and HEL cells. *Exp. Hematol.* 15:928.
16. Peetre, C., U. Gullberg, E. Nilsson, and I. Olsson. 1986. Effects of recombinant tumor necrosis factor, lymphotoxin and immune interferon on proliferation and differentiation of leukemic and normal hemopoietic precursor cells. *Exp. Hematol.* 14:131.
17. Murphy, M., B. Perussia, and G. Trinchieri. 1988. Effects of recombinant tumor necrosis factor, lymphotoxin and immune interferon on proliferation and differentiation of enriched hematopoietic precursor cells. *Exp. Hematol.* 16:131.
18. Oster, W., A. Lindemann, S. Horn, R. Mertelsmann, and F. Hermann. 1987. Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF).

- 1) by human monocytes. *Blood* 70:1700.
19. Okusawa, S., J. A. Gelfland, T. Ikejiryo, R. J. Connolly, and C. A. Dinarello. 1988. Interleukin-1 induces a shock like state in rabbits: synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J. Clin. Invest.* 81:1162.
20. Gray, P. W., B. B. Aggarwal, C. V. Benton, T. S. Bringman, W. J. Henzel, J. A. Jarrett, D. L. Leung, B. Moffat, P. Ng, L. P. Sverdrup, M. A. Palladino, and G. E. Nedwin. 1984. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis activity. *Nature* 312:721.
21. Granger, G. A., I. Masunaka, B. Averbook, M. Kobayashi, M. Fitzgerald, and R. Yamamoto. 1988. Differences in the bioactivity of recombinant human TNF, LT and T-cell derived LT-3 on transformed cells in vitro and the Meth A tumor growing in BALB/c mice. *Lymphokine Res.* 7:488.
22. Bevan, M., B. S. Anderson, P. Kelleher, F. Whalen, K. McCredie, and J. Guterman. 1987. Diversity of effect of recombinant tumor necrosis factors  $\alpha$  and  $\beta$  on human myelogenous leukemia cell lines. *Blood* 69:721.
23. Locksley, R. M., F. P. Heinzel, H. M. Shepard, J. Agosti, T. E. Eessalu, B. H. Aggarwal, and J. M. Harlan. 1987. Tumor necrosis factors  $\alpha$  and  $\beta$  differ in their capacities to generate interleukin-1 release from human endothelial cells. *J. Immunol.* 139:1891.
24. Broady, V. C., J. M. Harlan, and J. W. Adamson. 1987. Disparate effects of tumor necrosis factor  $\alpha$ /cachectin and tumor necrosis factor  $\beta$ /lymphotoxin on hematopoietic growth factor production and neutrophil adhesion molecule expression by cultured human endothelial cells. *J. Immunol.* 138:4298.
25. Koeffler, H. P., J. Gasson, J. Ranyard, L. Souza, M. Shephard, and R. Munko. 1987. Recombinant human TNF stimulates production of granulocyte colony-stimulating factor. *Blood* 70:55.
26. Shalaby, M. R., T. Espenik, G. C. Rice, A. J. Ammann, I. S. Figari, G. E. Ranges, and M. A. Palladino. 1988. The involvement of human tumor necrosis factors  $\alpha$  and  $\beta$  in the mixed lymphocyte reaction. *J. Immunol.* 141:499.
27. Averbook, B. J., R. S. Yamamoto, T. R. Ulich, E. W. B. Jeffes, I. Masunaka, and G. A. Granger. 1987. Purified native and recombinant human alpha lymphotoxin [tumor necrosis factor (TNF)-beta] induces inflammatory reactions in normal skin. *J. Clin. Immunol.* 7:333.
28. Aggarwal, B. B., T. E. Eessalu, and P. E. Hass. 1985. Characterization of receptors for human tumor necrosis factor and their regulation by  $\gamma$ -interferon. *Nature* 318:665.
29. Hass, P. E., A. Hotchkiss, M. Mohler, and B. Aggarwal. 1985. Characterization of specific high affinity receptors for human tumor necrosis factor on mouse fibroblasts. *J. Biol. Chem.* 260:12214.
30. Coffman, F. D., L. M. Green, and C. F. Ware. 1988. The relationship of receptor occupancy to the kinetics of cell death mediated by tumor necrosis factor. *Lymphokine Res.* 4:371.
31. Gullberg, U., M. Lantz, E. Nilsson, C. Peetre, G. Adolf, and I. Olsson. 1987. Characterization of a relationship between the T-lymphocyte derived differentiation inducing factor (DIF) and lymphotoxin: a common receptor system for DIF, lymphotoxin and tumor necrosis factor downregulated by phorbol esters. *Eur. J. Haematol.* 39:241.
32. Stauber, G. B., and B. B. Aggarwal. 1989. Characterization and affinity cross-linking of receptors for human recombinant lymphotoxin (tumor necrosis factor- $\beta$ ) on a human histiocytic lymphoma cell line, U937. *J. Biol. Chem.* 264:3573.
33. Hirano, K., K. Yamamoto, Y. Kobayashi, and T. Osawa. 1989. Characterization of specific high-affinity receptor for human lymphotoxin. *J. Biochem.* 105:120.
34. Marmenout, A., L. Franson, J. Travernier, J. van der Heyden, R. Tizard, E. Kawashima, A. Shaw, M-J. Johnson, D. Semon, R. Mueller, M-R. Ruysschaert, A. van Vilhet, and W. Fiers. 1985. Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. *Eur. J. Biochem.* 152:515.
35. Mossman, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65:55.
36. Rodbard, D., and G. R. Frazier. 1975. Statistical analysis of radioligand assay data. *Methods Enzymol.* 37:3.
37. Aggarwal, B. B., W. J. Henzel, B. Moffat, W. J. Kohr, and R. N. Harkins. 1985. Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. *J. Biol. Chem.* 260:2334.
38. Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* 262:10035.
39. Tschachler, E., M. Robert-Guroff, R. C. Gallo, and M. S. Reitz. 1989. Human T-lymphotrophic virus I-infected T cells constitutively express lymphotoxin in vitro. *Blood* 73:194.
40. Wingsfield, P., R. H. Pain, and S. Craig. 1987. Tumor necrosis factor is a compact trimer. *FEBS Lett.* 211:179.
41. Smith, R. A., and C. Baglioni. 1987. The active form of tumor necrosis factor is a trimer. *J. Biol. Chem.* 262:6951.
42. Aggarwal, B. B., B. Moffat, and R. N. Harkins. 1984. Human lymphotoxin: production by a lymphoblastoid cell line, purification and initial characterization. *J. Biol. Chem.* 259:686.
43. Kramer, S. M., M. E. Carver, and S. Apperson. 1986. Comparison of TNF- $\alpha$  and TNF- $\beta$  cytolytic biological activities in a serum free bioassay. *Lymphokine Res.* 5:S139.
44. Lee, S. H., B. B. Aggarwal, E. Rinderknecht, F. Assisi, and H. Chiu. 1984. The synergistic anti-proliferative effect of  $\gamma$ -interferon and human lymphotoxin. *J. Immunol.* 133:1083.
45. Stone-Wolf, D. S., Y. K. Yip, H. C. Keller, J. Le, Heniksen-Destanfano, B. Y. Rubin, E. Rinderknecht, B. B. Aggarwal, and J. Vilcek. 1984. Interrelationships of human interferon- $\gamma$  with lymphotoxin and monocyte cytotoxin. *J. Exp. Med.* 159:828.
46. Ruggiero, V., J. Travernier, W. Fiers, and C. Baglioni. 1986. Induction of the synthesis of tumor necrosis factor receptors by interferon- $\gamma$ . *J. Immunol.* 136:2445.
47. Aggarwal, B., and T. E. Eessalu. 1987. Induction of receptors for tumor necrosis factor- $\alpha$  by interferons is not a major mechanism for their synergistic cytotoxic response. *J. Biol. Chem.* 262:10000.
48. Calvo, J. C., J. P. Radicella, and E. H. Charreau. 1983. Measurement of specific radioactivities in labelled hormones by self-displacement analysis. *Biochem. J.* 212:259.
49. Tsujimoto, M., Y. K. Yip, and J. Vilcek. 1985. Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. *J. Immunol.* 132:7626.
50. Cantor, C. R., and P. R. Schimmel. 1980. The behavior of biological macromolecules. In *Biophysical Chemistry*. Part III. Freeman and Co., San Francisco, p. 860.
51. Imamura, K., D. Spriggs, and D. Kufe. 1987. Expression of tumor necrosis factor receptors on human monocytes and internalization of receptor bound ligand. *J. Immunol.* 139:2989.
52. Ishikra, H., K. Hori, and A. Bloch. 1989. Differential effects resulting from bimodal binding of recombinant human tumor necrosis factor to myeloid leukemia cells. *Blood* 73:419.
53. Kull, F. C., S. Jacobs, and P. Cuatrecasas. 1985. Cellular receptor for  $^{125}$ I-labeled tumor necrosis factor: specific binding, affinity labeling and relationship to sensitivity. *Proc. Natl. Acad. Sci. USA* 82:5756.
54. Creasey, A. A., R. Yamamoto, and C. R. Vitt. 1987. A high molecular weight component of human tumor necrosis factor receptor is associated with cytotoxicity. *Proc. Natl. Acad. Sci. USA* 84:3293.
55. Scheurich, P., E. Thoma, U. Uecker, and K. Pfizenmaier. 1987. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)- $\alpha$ : induction of TNF receptors on human T cells and TNF- $\alpha$  mediated enhancement of T cell responses. *J. Immunol.* 138:1786.
56. Chouaib, S., J. Bertoglio, J-Y. Blay, C. Marchiol-Fournigault, and D. Fradelizi. 1988. Generation of lymphokine activated killer cells: synergy between tumor necrosis factor and interleukin-2. *Proc. Natl. Acad. Sci. USA* 85:6875.
57. Ranges, G. E., M. P. Bombara, R. A. Aiyer, G. G. Rice, and M. A. Palladino. 1989. Tumor necrosis factor- $\alpha$  as a proliferative signal for an IL-2 dependent T cell line: strict species specificity of action. *J. Immunol.* 142:1203.
58. Plaetinck, G., W. Declercq, J. Travernier, M. Nabholz, and W. Fiers. 1987. Recombinant tumor necrosis factor can induce interleukin-2 receptor expression and cytolytic activity in a rat  $\times$  mouse T cell hybrid. *Eur. J. Immunol.* 17:1835.
59. Franson, L., M-R. Ruysschert, J. v.d. Heyden, and W. Fiers. 1986. Recombinant tumor necrosis factor: species specificity for a variety of human and murine transformed cell lines. *Cell. Immunol.* 100:260.
60. Stauber, G. B., R. A. Aiyer, and B. B. Aggarwal. 1988. Human tumor necrosis factor- $\alpha$  receptor: purification by immunoaffinity chromatography and initial characterization. *J. Biol. Chem.* 263:19098.
61. Kobayashi, Y., D. Miyaamotu, M. Asada, M. Obinata, and T. Osawa. 1986. Cloning and expression of human lymphotoxin mRNA derived from a human T-cell hybridoma. *J. Biochem.* 100:727.
62. Ruddle, N. H., and R. Homer. 1988. The role of lymphotoxin in the inflammatory response. *Prog. Allergy* 40:162.
63. Schaegger, H., and G. Jagow. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* 166:368.

# THE JOURNAL OF IMMUNOLOGY®

Official Journal of The American  
Association of Immunologists



HEALTH SCIENCES LIBRARY  
University of Wisconsin

SEP 27 1989

1305 Linden Dr.  
Madison, Wis. 53706

# Contents

---

## CELLULAR IMMUNOLOGY

---

- B. A. Araneo, T. Dowell, H. B. Moon, and R. A. Daynes 1737 Regulation of Murine Lymphokine Production in Vivo. Ultraviolet Radiation Exposure Depresses IL-2 and Enhances IL-4 Production by T Cells Through an IL-1-Dependent Mechanism
- W. Bartlett, A. Michael, J. McCann, D. Yuan, E. Claassen, and R. J. Noelle 1745 Cognate Interactions Between Helper T Cells and B Cells. II. Dissection of Cognate Help by Using a Class II-Restricted, Antigen-Specific, IL-2-Dependent Helper T Cell Clone
- R. H. Carter, and D. T. Fearon 1755 Polymeric C3dg Primes Human B Lymphocytes for Proliferation Induced by Anti-IgM
- N. K. Damle, and L. V. Doyle 1761 Stimulation via the CD3 and CD28 Molecules Induces Responsiveness to IL-4 in CD4<sup>+</sup>CD29<sup>+</sup>CD45R<sup>-</sup> Memory T Lymphocytes
- J. R. Drake, E. A. Repasky, and R. B. Bankert 1768 Endocytosis of Antigen, Anti-Idiotype, and Anti-Immunglobulin Antibodies and Receptor Re-Expression by Murine B-Cells
- H. G. Durkin, S. M. Chice, E. Gaetjens, H. Bazin, L. Tarcay, and P. Dukor 1777 Origin and Fate of IgE-Bearing Lymphocytes. II. Modulation of IgE Isotype Expression on Peyer's Patch Cells by Feeding with Certain Bacteria and Bacterial Cell Wall Components or by Thymectomy
- B. D. Evavold, and J. Quintans 1784 Accessory Cell Function of Th2 Clones
- H. Kizaki, T. Tadakuma, C. Odaka, J. Muramatsu, and Y. Ishimura 1790 Activation of a Suicide Process of Thymocytes Through DNA Fragmentation by Calcium Ionophores and Phorbol Esters
- S. MacPhail, and O. Stutman 1795 Specific Neonatal Induction of Functional Tolerance to Allogeneic M1s Determinants Occurs Intrathymically and the Tolerant State is M1s Haplotype-Specific
- D. J. McConkey, P. Hartzell, J. F. Amador-Pérez, S. Orrenius, and M. Jondal 1801 Calcium-Dependent Killing of Immature Thymocytes by Stimulation Via the CD3/T Cell Receptor Complex
- R. J. Noelle, J. McCann, L. Marshall, and W. C. Bartlett 1807 Cognate Interactions between Helper T Cells and B Cells. III. Contact-Dependent, Lymphokine-Independent Induction of B Cell Cycle Entry by Activated Helper T Cells
- L. F. Thompson, J. M. Ruedi, A. Glass, M. G. Low, and A. H. Lucas 1815 Antibodies to 5'-Nucleotidase (CD73), a Glycosyl-Phosphatidylinositol-Anchored Protein, Cause Human Peripheral Blood T Cells to Proliferate
- J. White, M. Blackman, J. Bill, J. Kappler, P. Marrack, D. P. Gold, and W. Born 1822 Two Better Cell Lines for Making Hybridomas Expressing Specific T Cell Receptors

---

## CLINICAL IMMUNOLOGY • IMMUNOPATHOLOGY

---

- J.-L. Pasquali, T. Martin, A.-M. Knapp, H. Levallois, and A. Farradji 1826 Monoclonal Rheumatoid Factor-Secreting Cells in a Patient With Mixed Cryoglobulinemia. Homogeneity and Stability of the Idiotypic Production and In Vitro Idiotypic Suppression
- C. Thiriart, M. Francotte, J. Cohen, C. Collignon, A. Declerq, S. Kummert, C. Molitor, D. Gilles, P. Roelants, F. van Wijndaele, M. De Wilde, and C. Bruck 1832 Several Antigenic Determinants Exposed on the GP120 Moiety of HIV-1 GP160 are Hidden on the Mature GP120

Continued on page 4

- B. Torok-Storb, L. Graf, and P. Shankar 1837 Inhibition of Erythroid Progenitor Growth is Mediated by Cytotoxic Lymphocytes and Not by Natural Killer Cells or Inf- $\gamma$
- A. H. Weller, K. Simpson, M. Herzum, N. Van Houten, and S. A. Huber 1843 Coxsackievirus-B3-Induced Myocarditis: Virus Receptor Antibodies Modulate Myocarditis

---

#### CYTOKINES • MEDIATORS • REGULATORY MOLECULES

---

- W. P. Arend, F. G. Joslin, R. C. Thompson, and C. H. Hannum 1851 An IL-1 Inhibitor from Human Monocytes. Production and Characterization of Biologic Properties
- J. Browning, and A. Ribolini 1859 Studies on the Differing Effects of Tumor Necrosis Factor and Lymphotoxin on the Growth of Several Human Tumor Lines
- J. H. Kehrl, A. S. Taylor, G. A. Delsing, A. B. Roberts, M. B. Sporn, and A. S. Fauci 1868 Further Studies of the Role of Tumor Growth Factor- $\beta$  in Human B Cell Function
- W. A. Massey, T. C. Randall, A. Kagey-Sobotka, J. A. Warner, S. M. MacDonald, S. Gillis, A. C. Allison, and L. M. Lichtenstein 1875 Recombinant Human IL-1 $\alpha$  and -1 $\beta$  Potentiate IgE-Mediated Histamine Release from Human Basophils
- T. Nishihara, Y. Ishihara, T. Noguchi, and T. Koga 1881 Membrane IL-1 Induces Bone Resorption in Organ Cultures
- P. N. Redlich, and S. E. Grossberg 1887 Analysis of Antigenic Domains on Natural and Recombinant Human IFN- $\beta$  by the Inhibition of Biologic Activities with Monoclonal Antibodies
- J.-C. Renaud, A. Vink, and J. Van Snick 1894 Accessory Signals in Murine Cytolytic T Cell Responses. Dual Requirement for IL-1 and IL-6

---

#### IMMUNOCHEMISTRY

---

- P. Anderson, M.-L. Blue, C. O'Brien, and S. F. Schlossman 1899 Monoclonal Antibodies Reactive With the T Cell Receptor  $\zeta$  Chain: Production and Characterization Using a New Method
- C. Boyer, N. Auphan, J. Gabert, D. Blanc, B. Malissen, and A.-M. Schmitt-Verhulst 1905 Comparison of Phosphorylation and Internalization of the Antigen Receptor/CD3 Complex, CD8, and Class I MHC-Encoded Proteins on T Cells
- Y. Nojima, S. Minota, A. Yamada, and F. Takaku 1915 Identification of an Acidic Ribosomal Protein Reactive with Anti-Sm Autoantibody
- R. Ono, K. Sakaguchi, and S. Ferrone 1921 Modulation by Antidiidotypic Monoclonal Antibodies of Immune Lysis Mediated by Anti-HLA Monoclonal Antibodies
- R. Pulido, and F. Sánchez-Madrid 1930 Biochemical Nature and Topographic Localization of Epitopes Defining Four Distinct CD45 Antigen Specificities. Conventional CD45, CD45R, 180 kDa (UCHL 1) and 220/205/190 kDa
- C. J. Thaler, W. P. Faulk, and J. A. McIntyre 1937 Soluble Antigens of IgE Receptor Fc $\gamma$ RIII in Human Seminal Plasma
- L. S. Zalman, M. A. Brothers, and H. J. Müller-Eberhardt 1943 Isolation of Homologous Restriction Factor from Human Urine. Immunological Properties and Biologic Activity

---

#### IMMUNOPHARMACOLOGY

---

- T. D. Bigby, and N. Meslier 1948 Transcellular Lipoxygenase Metabolism between Monocytes and Platelets
- F. Di Virgilio, E. Bronte, D. Collavito, and P. Zanovello 1955 Responses of Mouse Lymphocytes to Extracellular Adenosine 5'-Triphosphate (ATP). Lymphocytes with Cytotoxic Activity Are Resistant to the Permeabilizing Effects of ATP

Continued from page 4

- C. Kreis, M. La Fleur, C. Ménard, R. Paquin, and A. D. Beaulieu 1961 Thromb spondin and Fibronectin are Synthesized by Neutrophils in Human Inflammatory Joint Disease and in a Rabbit Model of In Vivo Neutrophil Activation
- R. Kurrasch, F. Elfman, and H. D. Perez 1969 Polymorphonuclear Leukocytes CAP A Derivative of Wheat Germ Agglutinin Upon Stimulation with Formyl Peptide and C5a but Not Leukotriene B<sub>4</sub>
- J. P. Messina, and D. A. Lawrence 1974 Cell Cycle Progression of Glutathione-Depleted Human Peripheral Blood Mononuclear Cells is Inhibited at S Phase
- H. Takizawa, K. Ohta, K. Hirai, Y. Misaki, T. Horiuchi, N. Kobayashi, J. Shiga, and T. Miyamoto 1982 Mast Cells are Important in the Development of Hypersensitivity Pneumonitis. A Study with Mast-Cell-Deficient Mice
- N. Topley, J. Floege, K. Wessel, R. Hass, H. H. Radeke, V. Kaever, and K. Resch 1989 Prostaglandin E<sub>2</sub> Production is Synergistically Increased in Cultured Human Glomerular Mesangial Cells by Combinations of IL-1 and Tumor Necrosis Factor- $\alpha$
- K. A. Yamaoka, H.-E. Claesson, and A. Rosén 1996 Leukotriene B<sub>4</sub> Enhances Activation, Proliferation, and Differentiation of Human B Lymphocytes

---

#### MICROBIAL IMMUNOLOGY

---

- E. Cillari, M. Dieli, E. Maltese, S. Milano, A. Salerno, and F. Y. Liew 2001 Enhancement of Macrophage IL-1 Production by *Leishmania major* Infection In Vitro and Its Inhibition by IFN- $\gamma$
- D. P. Harris, B. T. Backstrom, R. J. Booth, S. G. Love, D. R. Harding, and J. D. Watson 2006 The Mapping of Epitopes of the 18-kDa Protein of *Mycobacterium leprae* Recognized by Murine T Cells in a Proliferation Assay
- L. S. Klavinskis, A. Tishon, and M. B. Oldstone 2013 Efficiency and Effectiveness of Cloned Virus-Specific Cytotoxic T Lymphocytes In Vivo
- S. Kumar, M. F. Good, F. Dontaifraid, J. M. Vinetz, and L. H. Miller 2017 Interdependence of CD4<sup>+</sup> T Cells and Malarial Spleen in Immunity to *Plasmodium vivax*. Relevance to Vaccine Development
- A. Kurata, T. J. Parker, R. D. Streilein, R. M. Scearce, B. F. Haynes, and J. A. Berzofsky 2024 Immunodominant Sites of Human T-Cell Lymphotropic Virus Type 1 Envelope Protein for Murine Helper T-Cells
- F. C. Rawle, A. E. Tollefson, W. S. M. Wold, and L. R. Gooding 2031 Mouse Anti-Adenovirus Cytotoxic T Lymphocytes. Inhibition of Lysis by E3 gpl9K but Not E3 14.7K
- H. L. Shear, R. Srinivasan, T. Nolan, and C. Ng 2038 Role of IFN- $\gamma$  in Lethal and Nonlethal Malaria in Susceptible and Resistant Murine Hosts
- Y. Suzuki, F. K. Conley, and J. S. Remington 2045 Importance of Endogenous IFN- $\gamma$  for Prevention of Toxoplasmic Encephalitis in Mice
- M. Yasukawa, A. Inatsuki, and Y. Kobayashi 2051 Differential In Vitro Activation of CD4<sup>+</sup>CD8<sup>-</sup> and CD8<sup>+</sup>CD4<sup>-</sup> Herpes Simplex Virus-Specific Human Cytotoxic T Cells

---

#### MOLECULAR BIOLOGY • MOLECULAR GENETICS

---

- C. B. Kurtz, M. S. Paul, M. Aegester, J. J. Weis, and J. H. Weis 2058 Murine Complement Receptor Gene Family. II. Identification and Characterization of the Murine Homolog (Cr2) to Human CR2 and Its Molecular Linkage to Cr3y
- P. Merryman, J. Silver, P. K. Gregersen, G. Solomon, and R. Winchester 2068 A Novel Association of DQ $\alpha$  and GQ $\beta$  Gene in the DRw10 Haplotype. Determination of a DQw1 Specificity by the DQ  $\beta$ -Chain
- S. Rath, J. Durdik, R. M. Gerstein, E. Selsing, and A. Nisonoff 2074 Quantitative Analysis of Idiotypic Mimicry and Allelic Exclusion in Mice with a  $\mu$  Ig Transgene

Continued on page 6

<b>Erratum</b>	<b>2081</b>
<b>Announcements</b>	<b>2081</b>
<b>Author Index</b>	<b>2082</b>